ANNEX 4

The interpretation of indicators of iron status during an acute phase response

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Contents

1. Abstract

Iron status is influenced by infection or trauma. The objective of this paper is to describe how indices of iron status, particularly the concentration of ferritin and soluble transferrin receptor (TfR), are influenced by changes in the concentration of acute phase proteins (APPs) during infection or trauma. Measurements of the change in concentration of APPs after elective surgery that are not preceded by infection are used to show the difference in responses of these proteins. Changes in the concentration of APPs and markers of iron status during treatment for infection are also used to demonstrate the interrelationship between indicators. In developing countries asymptomatic malaria is common and produces an acute phase response, but the data on the concentration of APPs and indices of iron status in asymptomatic malaria are inconclusive with respect to using either serum TfR or ferritin as a marker of iron status in such situations. In individuals infected with human immunodeficiency virus (HIV) there may be an atypical acute phase response in the absence of opportunistic infections. Some tentative conclusions are drawn concerning the interrelationships between ferritin and two APPs, C-reactive protein (CRP) and α-1-acid glycoprotein (AGP), during an acute phase response.

2. Introduction

Ferritin concentration is an important indicator of total body iron stores and a concentration of <12–15 µg/l is taken to indicate deficient iron stores (*1*). However, the synthesis of ferritin is stimulated by infection, which may either obscure an iron deficiency or indicate a larger iron store than truly exists. In this paper, the nature of the acute phase response and its relationship to iron metabolism is illustrated.

Iron deficiency is the most common micronutrient deficiency in the world. It can affect all populations and age groups, but the most vulnerable groups are women and children. Anaemia is commonly used as an indicator of iron deficiency in population-based surveys, but iron deficiency is not the only cause of anaemia: infections, haemoglobinopathies, and vitamin A deficiency can all lead to anaemia. A high prevalence of anaemia is often found in developing countries, especially where infections such as malaria or hookworm are common. In addition infection with HIV is affecting millions of people in the developing world and may influence their iron status, but little is known about the acute phase response during HIV infection in the absence of opportunistic infection.

3. The acute phase response

Infection and trauma are accompanied by an acute phase response, a non-specific process that includes the production of APPs prior to the full activation of the immune response. The main purpose of the acute phase response is to prevent damage to tissues, and remove harmful molecules and pathogens. During such a response the concentration of some APPs, called positive APPs, increase in the plasma and others, called negative APPs, decrease. The changes in the concentrations of APPs are due largely to changes in their production by hepatocytes, which in turn are regulated by cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor α (TNF-α), which act in a complex network (*2*).

The role of the positive APPs includes host-adaptive and host-defense mecha-

nisms, which act by binding to foreign substances and by modulating phagocytic cell functions. The positive APPs include CRP, α-1-antichymotrypsin (ACT), AGP, also known as orosomucoid, serum amyloid A (SAA), fibrinogen, haptoglobin, caeruloplasmin and ferritin. An increase in serum ferritin concentration occurs in response to any infectious or inflammatory process, but serum ferritin concentration also reflects total body iron stores, hence a low serum ferritin concentration can only reflect depleted iron stores in the absence of infection. The magnitude of the change in concentration of the APPs during an acute phase response varies considerably: caeruloplasmin can increase by about 50% whereas CRP can increase by as much as 1000-fold (*3*,*4*). The APPs that decrease in concentration include transferrin, albumin, transthyretin and retinol binding protein (RBP). These proteins are not thought to have an immune function, but rather to act as transport proteins and as a result, the plasma concentration of the specific nutrients they carry may be reduced during infection and inflammation (*4*).

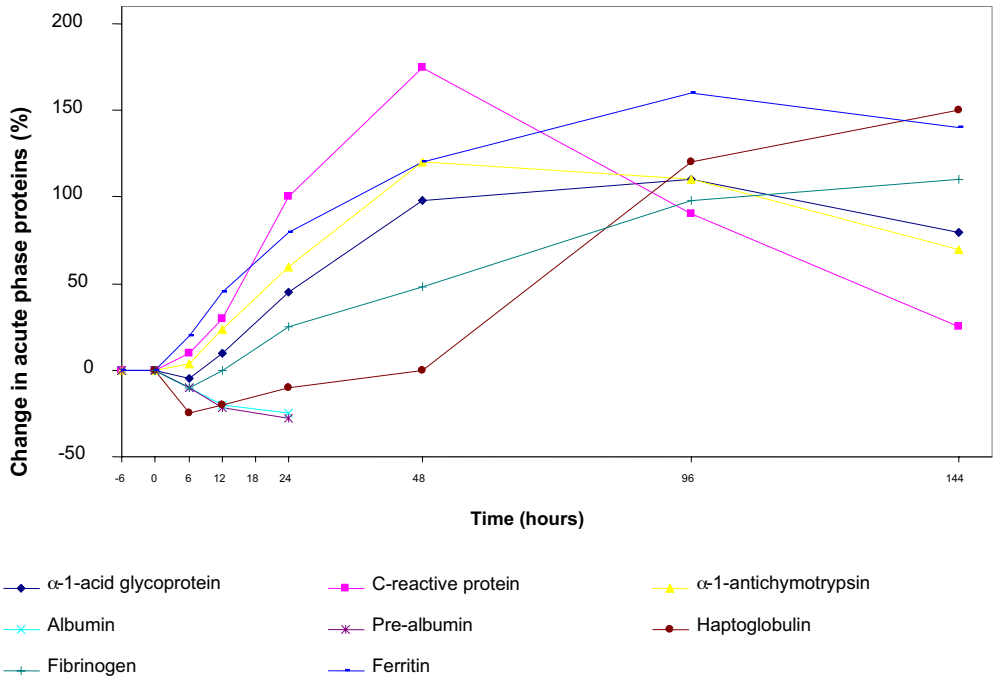
4. Sequence of events

The concentration of APPs after elective surgery that is not preceded by infection can be used to demonstrate the time course over which their production is stimulated. The first change during the acute phase response is in cortisol concentration, which peaks at 6 hours after surgery, followed by a rise in the number of white blood cells, which peak at 10 hours. Data obtained after cholecystectomy indicate that CRP and ACT rise rapidly in the first 6 hours and continue to rise for the first 20 hours after surgery, whereas AGP shows only a small increase during the same time period (Figure 1). Albumin and pre-albumin both decrease in concentration. Haptoglobulin initially decreases but returns to its initial concentration by 20 hours before showing a steady increase in concentration over the next few days (*4*).

Data collected over 6 days following elective surgery showed that CRP rises very rapidly from normal concentrations of <5 mg/l to concentrations of >100 mg/l, peaking at day 2, and then slowly decline. Fibrinogen and AGP show a slower initial rise but do not return to a normal concentration until up to a week after CRP had returned to normal (*4*). Data from Fleck and Myers (*4*), however, showed that the rise in concentration of AGP and fibrinogen was preceded by a slight fall in concentration immediately after the operation. An initial decrease in AGP was also observed by Myers et al. (*5*) 2–4 hours after making a skin incision for a hysterectomy, and this preceded an increase in AGP at 6 hours. The initial decrease in AGP concentration is thought to be due to changes in microvascular permeability immediately following trauma which may facilitate the movement of proteins between the plasma and damaged tissues (*3*).

Data on the concentration of CRP, SAA, haptoglobulin, fibrinogen, and albumin collected over a period of 21 days after surgery confirmed and extended the information from the first study (*4*). Both CRP and SAA increased rapidly and reached a peak concentration between 48–72 hours after surgery. With no post-operative complications, the concentration of CRP returned to normal by the 10th post-operative day, whereas SAA did not return to normal values until about 12–14 days after the operation. In contrast, haptoglobulin and fibrinogen initially decreased in concentration, then slowly increased, and only achieved maximum concentrations at 10 and 12 days, respectively; fibrinogen returned to normal, but the concentration of haptoglobulin was still higher than normal at day 21 (*3*).

FIGURE 1
Characteristic patterns of change in acute phase proteins (APPs) after trauma. APPs are shown as percent change from the initial concentration.



Adapted from Feelders et al. (*2*), Gabay and Kushner (*3*), Fleck and Myers (*4*), Wieringa et al. (*6*), with permission of the publishers.

5. Changes in acute phase indicators during treatments for various diseases

Baynes et al. (*7*) reported a number of small studies in which they followed the changes in concentration of various acute phase indices in patients with different illnesses, and showed sequential changes during treatment.

The increase in plasma ferritin concentration paralleled the increase in plasma CRP during acute pneumonia, tuberculosis, rheumatoid arthritis and neutropenic sepsis, suggesting that ferritin was acting as an APP. However, the degree to which the ferritin concentration rose was influenced by the underlying iron status of the subjects. The plasma ferritin concentration was found to be much lower in individuals with rheumatoid arthritis, who were iron deficient as well as being chronically ill, whereas the concentration of CRP always seemed to be increased in proportion to the severity of disease.

In neutropenic sepsis, both CRP and ferritin were markedly increased in concentration. During treatment there was a sharp drop in CRP concentration that was not paralleled by a drop in plasma ferritin concentration. The different responses may reflect different turnover rates, with CRP concentration responding rapidly to the removal of the trauma (i.e. the chemotherapy), whereas the ferritin concentration declined more slowly, perhaps because erythropoiesis was still depressed by the production of cytokines (*7*).

6. Acute phase proteins, iron indicators and the influence of cytokines

A low serum iron concentration and a high serum ferritin concentration during an acute phase response are associated with a redistribution of iron into the liver and mononuclear phagocyte system, both mediated by cytokines (*2*). In experimental animals the administration of the cytokines TNF-α, IL-1 and IL-6 induced a decrease in serum iron concentration within 3–6 hours and in similar studies *in vitro*, cytokines induced an increase in ferritin synthesis (*2*). Feelders et al. (*2*) carried out an experiment in humans and reported similar results to those found *in vitro* and in experimental animals, i.e. the administration of cytokines resulted in hypoferraemia associated with an increased ferritin production.

In the study by Feelders et al. (*2*) 12 patients with inoperable soft-tissue sarcoma or melanoma were treated for 2 days with the T-cell-derived cytokine human interferon γ (IFN γ), which is thought to prime activated macrophages. Serum samples were taken on two days before treatment and at baseline. All patients were then cannulated, an isolated limb perfusion was performed using recombinant human TNF-α and human IFN γ, and further blood samples were taken for 7 days after treatment. Although an acute phase response occurs in many types of cancer (*8*,*9*) and would be expected to produce associated changes in iron metabolism, Feelders et al. (*2*) showed that APPs (except the concentration of AGP, which was slightly higher than normal) and iron status were normal before treatment, suggesting that the local tumours were not producing a systemic reaction.

Following treatment with recombinant TNF-α and IFN γ, all APPs except CRP and ferritin decreased in concentration, probably due to haemodilution and capillary leakage. Both CRP and ferritin increased in concentration after the start of perfusion, representing an early acute phase response, while AGP responded more slowly in a second response. The concentration of CRP showed a sharp decrease after peaking at day 2; ferritin decreased slowly in concentration but was still higher than normal at day 7, as was AGP. In contrast the two negative APPs, albumin and transferrin, decreased in concentration from the first pre-treatment day, reached a nadir at the baseline sample, then remained low for 2 more days before slowly increasing in concentration. Serum iron and serum TfR concentrations decreased during pretreatment, and remained low after perfusion, with the lowest concentrations recorded 8 hours and 1 day afterwards, respectively.

The experiment showed that the administration of TNF in humans caused hypoferraemia associated with an increased ferritin production. The data also confirm a previous report that the increase in ferritin concentrations parallels that of CRP (*7*), suggesting that ferritin responds as an early APP. However the plasma ferritin concentration remained high for longer than the CRP concentration thus, in the latter part of the experiment, ferritin behaved more like AGP.

7. Control of iron metabolism

The regulation of iron metabolism is normally under the control of iron regulatory proteins (IRPs) that bind to sequences on messenger ribonucleic acid (mRNA) and protect mRNA from degradation. As a consequence of iron deficiency the IRPs bind to mRNA which promotes the expression of transferrin receptor protein and represses the synthesis of ferritin. When iron is present in adequate amounts, ferritin syn-

thesis is promoted and iron storage occurs. During infection the normal control of iron metabolism is changed by IL-1 and TNF-α. The plasma ferritin concentration increases, despite a low concentration of serum iron, because ferritin mRNA is more sensitive to cytokines than to iron (*10*).

The low concentration of serum iron found during infection (hypoferraemia) is accompanied by changes in the plasma concentration of several iron-binding proteins (Table 1) that facilitate iron uptake by the reticuloendothelial system of the gut or the removal and re-use of haemoglobin released from old erythrocytes (*10*).

TABLE 1
Influence of inflammation on iron-binding proteins in plasma

Plasma protein (units)	Normal range	Function	Change in plasma concentration in response to infection
Caeruloplasmin (g/l)	0.16–0.53	Converts Fe^{II} to Fe^{III}	Increases ~ 50%
Transferrin (g/l) (μmol/l)	1.9–2.58 25–34	Binds and transports iron	Decreases ~ 30%
Lactoferrin (μg/l)	0.91–0.45	Binds iron, especially at low pH	Released from granulocytes. Increases 200–500%
Ferritin (μg/l) (pmol/l)	15–250 34–562	Binds iron	Can increase 3000%
Haptoglobulin (g/l) (μmol/l)	0.70 – 3.79 7.0–37.9	Binds haemoglobin	Increases 200–500%

Adapted from Thurnham and Northrop-Clewes (*10*), with permission of the publisher.

Hypoferraemia may protect individuals against infection by withholding iron from pathogenic micro-organisms and by reducing the potential pro-oxidant properties of iron which may exacerbate tissue damage at the site of inflammation where reactive oxygen species are being produced and the body's cells are at risk of damage (*11*). One theory suggested that lactoferrin acts in the hypoferraemic-hyperferritinaemic response to inflammation by causing a drop in plasma iron concentration and a rise in plasma ferritin by removing iron from transferrin and delivering it to the macrophages, where it is bound to ferritin (*11*). However, it is now thought that lactoferrins exert antimicrobial properties that are independent of binding iron, although their mode of action has not been elucidated (*10*).

8. Ferritin

In clinical practice the gold standard to estimate iron stores is to stain a bone marrow aspirate for iron, but this is not practical to do during population surveys, so alternative methods have been sought. The World Health Organization recommends that a serum ferritin concentration <12 μg/l indicates depleted iron stores in children <5 years of age, while a concentration <15 μg/l indicates depleted iron stores in those >5 years of age (*1*). However, both thresholds may be too low during an acute phase response or when there is chronic disease, and a serum ferritin concentration between 30 and 100 μg/l may better indicate depleted iron stores in such circumstances (*1*,*12*).

The fact that the measurement of serum ferritin concentration can reflect the total body iron store and an acute phase response has been known since the 1970s, but the exact kinetics of the changes that occur are not known in detail (*2*). Understanding how to interpret the concentration of serum ferritin in the presence of infection

is difficult, and various approaches have been suggested. An early idea was to use the ferritin concentration and the mean corpuscular volume or haematocrit, which is low during anaemia, but it can also be altered by a wide variety of conditions including B vitamin deficiencies, liver, thyroid and kidney disease, and so was not a good choice. It became clear that to interpret the serum ferritin concentration some measure of the acute phase response was needed (*12*). Therefore it was suggested that, in addition to ferritin, an independent indicator of the acute phase response, such as CRP or AGP, should be measured (*13*). The report of the joint World Health Organization/Centers of Disease Control and Prevention technical consultation on the assessment of iron status at the population level proposes a meta-analysis of existing data to explore the possibility of using one or two APPs to correct serum ferritin concentration in the presence of infection.

Other approaches to help interpret the ferritin concentration in the presence of infection include: the determination of serum iron concentration with the percentage saturation of serum iron-binding capacity (transferrin), because a high serum ferritin concentration and a transferrin saturation <45% usually indicates infection (14); the measurement of TfR alone (discussed below) because it is thought to be unaffected by infection; and the measurement of TfR plus ferritin. However there is disagreement about the use of ferritin as well as TfR because it is thought that ferritin does not improve the diagnostic efficiency of measuring TfR alone, or that the calculation of the TfR/log ferritin ratio is more useful (*15*).

9. Serum transferrin receptor

Measuring the concentration of serum TfR is as an alternative method to assess iron status because the concentration increases during iron deficiency. It is thought that the serum TfR concentration is not increased in individuals during an acute phase response therefore the measurement of serum TfR may help to distinguish between individuals with and without iron deficiency in the presence of infection. However no international reference standard exists for this assay, and there may be difficulties in interpreting serum TfR in the presence of some chronic infections (*15*).

Transferrin is the main iron transport protein found in blood. It delivers iron to cells where it interacts with the specific membrane receptor, called TfR (*14*). Serum TfR is a truncated monomer of the tissue receptor, lacking the first 100 amino acids (*15*). Mean serum TfR concentrations tend to be in the range of 5–8 mg/l in normal subjects, but standards for different commercial assays vary and results cannot easily be compared.

Serum TfR concentrations can range from 8 times below to 20 times above normal values. The most important factor controlling this variation is bone marrow erythropoietic activity (*15*). Serum TfR concentrations indicate the absolute rate of erythropoiesis and the adequacy of marrow proliferative capacity for any level of anaemia. As the iron supply to the tissues becomes deficient the concentration of TfR on cell surfaces increases progressively and independently of the presence of adequate iron stores. This means that an increase in serum TfR concentration is a sensitive and quick response to the development of iron deficiency. Conversely, the serum TfR concentration decreases in response to treatment with iron before a change in haemoglobin occurs, so the response to iron can be monitored by changes in serum TfR (*15*).

The serum TfR concentration may be slightly increased to 9 mg/l in non-anaemic iron deficiency, but can be much higher (25 mg/l), in iron deficiency anaemia (IDA). The concentration of serum TfR is not increased if there is an acute phase response (Table 2) therefore serum TfR distinguishes between IDA and iron deficiency due to an acute phase response. The absence of an increase in TfR concentration during the anaemia of chronic disease (ACD) is due to the action of the cytokine IL-1, the primary mediator of the acute phase response. During ACD erythrocyte survival is somewhat reduced and IL-1 prevents the adequate release of iron from the reticuloendothelial stores so there is not enough iron for erythropoiesis. In conditions where there is ACD as well as IDA, then the serum TfR concentration may be increased to a similar degree as for IDA alone (Table 2).

TABLE 2
Differential diagnosis of iron deficiency anaemia (IDA), anaemia of chronic disease (ACD) and the combination of both, where N indicates no change in the concentration or ratios of indicators and the arrows indicate the direction of a change.

	Concentrations of:				Ratio of soluble transferrin receptor/ ferritin
	Haemoglobin	Serum iron	Ferritin	Soluble transferrin receptor	
Iron deficiency anaemia (IDA)	↓	↓	↓	↑	↑ ↑
Anaemia of chronic disease (ACD)	↓	↓	N-↓	N	N
ACD and IDA	↓	↓	↓	↑	↑

Adapted from Beguin (*15*), with permission of the publisher.

Unfortunately the serum TfR concentration may not always distinguish between patients with or without iron deficiency in the presence of some chronic conditions. For example, in some forms of ACD, the serum TfR concentration may remain normal even when IDA is present, because marrow erythropoietic activity may be suppressed by cytokines. Therefore the relationship between iron status and the serum TfR concentration in inflammation may be affected by the degree of anaemia but also, and more importantly, by the effect of the cytokines on erythropoietic activity. It has been proposed that the combined use of the concentration of serum TfR and ferritin, or the use of the ratio of the concentration of serum TfR/ferritin or serum TfR/log ferritin, may help to identify iron deficiency in patients with a chronic acute phase reaction (*16*,*17*). In particular, Beguin (*15*) suggests that the log (serum TfR/ ferritin) ratio may prove to be the most useful.

10. Asymptomatic malaria, acute phase proteins and iron

Assessing iron status in areas where malaria is endemic presents difficulties in symptomatic and apparently healthy individuals. Continual exposure to malaria parasites induces varying degrees of immunity in a population, such that asymptomatic and apparently healthy adults and older children have parasitaemia but no clinical disease. The presence of parasites may produce a chronic acute phase response, even in an asymptomatic individual, resulting in an elevated serum ferritin concentration. Asymptomatic malaria is also associated with a lower than normal haemoglobin concentration and an increased serum TfR concentration, which indicates that the presence of the parasite is associated with haemolysis.

Several studies support the finding of a raised serum TfR concentration in people

with asymptomatic malaria (*18–20*). However Stoltzfus et al. (18) found that although the serum TfR increased with parasite density in children, this increase disappeared when serum TfR was adjusted for the haemoglobin concentration. Furthermore, Verhoef et al. (*21*) suggest that, because of malaria-induced haemolysis, the serum TfR concentration may not be a useful measure of iron deficiency in individuals with malaria, and that further studies are needed to elucidate the relationship between the serum TfR concentration and malaria.

Stoltzfus et al. (*18*) found no relationship between the serum ferritin concentration and the density of malaria parasites when the concentration of parasites was <1000/µl blood, but above this parasite density the serum ferritin concentration was higher by 1.5 µg/l per 1000 parasites. Odunukwe et al. (22) have proposed that serum ferritin could be a useful marker of iron status during asymptomatic malaria. They suggested that there is a linear relationship between the serum ferritin concentration and malaria parasite density in apparently healthy adults, irrespective of the species of *Plasmodium*, and that the ferritin concentration can be corrected using the following formula:

Measured serum ferritin concentration – (0.08 µg * malaria density) = ferritin level (µg/l), where malaria density is measured in counts/µl blood.

The usefulness of this formula has not yet been confirmed.

11. Human immunodeficiency virus, acute phase proteins and iron

Infection with HIV is accompanied by a progressive accumulation of iron in macrophages, endothelial and other cells, and can result in an iron excess in the bone marrow, brain and other organs during the advanced stages of disease. The main cause of the iron excess is the chronic acute phase response which retains iron in the reticulo-endothlial system and acts to sustain a low serum iron concentration (*23*). A consequence of such iron loading is the growth of microorganisms, resulting in the opportunistic infections typical of acquired immunodeficiency syndrome (AIDS).

Because iron is needed for lymphocyte activation and proliferation, an altered immune function related to imbalances of iron metabolism might be a special problem in patients with HIV (*24*). The proliferative phase of lymphocyte activation requires iron because it is essential for enzymes such as ribonucleotide reductase, which is involved in deoxyribonucleic acid (DNA) synthesis (*24*). Changes in iron status appear to exert subtle effects on the immune system in HIV by altering the proliferation of T-lymphocytes and B-lymphocytes (*25*).

The results of a number of studies suggest that infection by HIV alone can elicit an APP response but, overall, the acute phase response in asymptomatic HIV is mild (*26–30*). In a study of children with HIV infection, without secondary infection, Jahoor et al. (*29*) found that the APP response may be different from the response elicited by bacterial infections because the higher concentrations and faster synthesis rates of the positive APPs were not accompanied by lower concentrations and synthesis rates of the negative APPs. The results from this study of children (*29*) confirmed previous data from the same author on asymptomatic HIV-infected adults. Their findings suggest that because the negative APPs are also transport proteins, the concentration of the specific nutrients they carry may not be reduced during early but asymptomatic HIV infection.

12. Summary

- An increased concentration of serum TfR is a good indicator of tissue iron deficiency, irrespective of iron stores.
- The concentration of serum TfR is not increased when anaemia is due to inflammation, so changes in the serum TfR concentration can distinguish between IDA and ACD. However, when IDA and ACD are both present, the serum TfR concentration is increased, irrespective of the acute phase response.
- In some chronic conditions erythropoietic activity may be increased even though there is no IDA and, in such circumstances, the concentration of serumTfR may be difficult to interpret. Various ways of using the ratio of serum TfR to serum ferritin have been suggested to help identify IDA in the presence of such a chronic acute phase response.
- Normally the synthesis of ferritin and TfR proteins is regulated post-transcriptionally by the intracellular iron concentration. However, an increased ferritin synthesis during an acute phase response appears to be stimulated by cytokines, independently of intracellular iron.
- Serum ferritin increases during an acute phase response, although the final concentration of ferritin is influenced by the underlying iron status.
- In the studies cited, ferritin behaved as a fast-acting positive APP at the beginning of the acute phase response and paralleled the changes in concentration of CRP. However in the later stages of the acute phase response, the concentration of ferritin remained high, and it behaved more like AGP.
- Thus, in the initial phase of infection the change in concentration of CRP may predict the behaviour of ferritin, and in the later stages AGP may control for the confounding effects of the acute phase response. Measurement of both these APPs may help to interpret the changes in serum ferritin concentration: if only the CRP concentration is elevated then the infection is in the initial stages; if both CRP and AGP are elevated in concentration then the infection is in the acute stage; and if only AGP is elevated then the infection is in the chronic stage and a correction factor to interpret ferritin in each stage could be calculated.
- More information is needed to interpret iron status as a result of an acute phase response. The report from the joint World Health Organization/Centers for Disease Control and Prevention technical consultation on the assessment of iron status at the population level suggests the use of a meta-analysis to explore the possibilities of using one or two APPs to correct ferritin in the presence of infection.

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